

WHAT IS CLAIMED IS:

1. A method for obtaining an isolated polynucleotide encoding an enhanced Rubisco protein having Rubisco catalytic activity wherein the K_m for CO_2 is significantly lower than a protein encoded by a parental polynucleotide encoding a naturally-occurring Rubisco enzyme, the method comprising:

recombining sequences of a plurality of parental polynucleotide species encoding at least one Rubisco sequence under conditions suitable for sequence shuffling to form a resultant library of sequence-shuffled Rubisco polynucleotides;

transferring said library into a plurality of host cells forming a library of transformants wherein sequence-shuffled Rubisco polynucleotides are expressed;

selecting for enhanced growth at low CO_2/O_2 ratios or assaying individual or pooled transformants for Rubisco catalytic activity to determine the relative or absolute K_m for CO_2 and thereby identifying at least one enhanced transformant that expresses a Rubisco activity which has a significantly lower K_m for CO_2 than the Rubisco activity encoded by the parental sequence(s);

recovering the sequence-shuffled Rubisco polynucleotide from at least one enhanced transformant.

2. The method of claim 1, further comprising the step of subjecting a recovered sequence-shuffled Rubisco polynucleotide encoding an enhanced Rubisco to at least one subsequent round of recursive shuffling and selection, wherein said recovered sequence-shuffled Rubisco polynucleotide is used as at least one parental sequence for subsequent shuffling.

3. The method of claim 1, wherein selection comprises assaying individual or pooled transformants for Rubisco catalytic activity to determine the relative or absolute K_m for O_2 and identifying at least one enhanced transformant that expresses a Rubisco activity which has a significantly higher K_m for O_2 than the Rubisco activity encoded by the parental sequence(s).

4. The method of claim 1, wherein selection comprises assaying individual or pooled transformants for Rubisco catalytic activity to determine the relative or absolute K_m for O_2 and K_m for CO_2 identifying at least one enhanced transformant that expresses a Rubisco activity which has a significantly lower ratio of K_m for CO_2 to K_m for O_2 than the Rubisco activity encoded by the parental sequence(s).

5. The method of claim 1, wherein selection comprises assaying samples of individual transformants and their clonal progeny which are isolated into discrete reaction vessels for Rubisco activity assay, or are assayed in situ.

6. The method of claim 1, wherein the host cell comprises a non-photosynthetic bacterium lacking an endogenous ribulose-5-phosphate kinase activity and is transformed with an expression cassette encoding the production of a functional ribulose-5-phosphate kinase ("R5PK") activity, thereby forming an R5PK host cell, optionally including an expression cassette encoding a complementing Rubisco S subunit and, wherein selection comprises culturing the population of transformed R5P host cells in the presence of labelled carbon dioxide and/or labelled bicarbonate for a suitable incubation period, determining the amount of labelled carbon that is fixed by each transformed host cell and its clonal progeny relative to the amount of carbon fixed by untransformed R5PK host cells cultured under equivalent conditions.

7. The method of claim 6, wherein the R5PK host cells harbor expression cassettes encoding a complementing an L subunit and the library comprises shuffled S subunit encoding sequences.

8. The method of claim 6, wherein the host cell is a strain of non-photosynthetic bacterium which lacks endogenous phosphoglycerate kinase (PGK) activity and harbors an expression cassette encoding R5P kinase (R5PK) forming a PGK(-)/R5PK host cell.

9. The method of claim 8, wherein the host cell encodes a complementing subunit, and the method comprises the further step of culturing the population of transformed R5PK host cells in a minimal growth medium including glucose, wherein the minimal medium including glucose is insufficient to support the growth and replication of an untransformed PGK-/R5PK host cell, but is sufficient to support the growth and replication of a transformed PGK-/R5PK host cell expressing a functional Rubisco carboxylase activity.

10. A plant cell protoplast and clonal progeny thereof containing a sequence-shuffled polynucleotide encoding a Rubisco subunit which is not encoded by the naturally occurring genome of the plant cell protoplast.

11. A collection of plant cell protoplasts transformed with a library of sequence-shuffled Rubisco subunit polynucleotides in expressible form.

12. A regenerated plant containing at least one species of replicable or integrated polynucleotide comprising a sequence-shuffled portion and encoding a Rubisco subunit polypeptide.

13. A regenerated plant containing a polynucleotide expression cassette encoding a marine algal *rbcL* gene.

14. A regenerated plant of claim 13, further comprising a polynucleotide expression cassette encoding a marine algal *rbcS* gene.

15. A polynucleotide comprising: (1) a sequence encoding a shuffled Rubisco Form I L subunit gene (*rbcL*) linked to (2) a selectable marker gene which affords a means of selection when expressed in chloroplasts, and, optionally, flanked by (3) an upstream flanking recombinogenic sequence having sufficient sequence identity to a chloroplast genome sequence to mediate efficient recombination and (4) a downstream flanking recombinogenic sequence having sufficient sequence identity

to a chloroplast genome sequence to mediate efficient recombination.

16. A polynucleotide of claim 15, wherein the polynucleotide encodes an enhanced Rubisco protein having Rubisco catalytic activity wherein the K_m for CO_2 is significantly lower than a protein encoded by a parental polynucleotide encoding a naturally-occurring Rubisco enzyme.

17. A polynucleotide of claim 15, wherein the polynucleotide encodes an enhanced Rubisco protein having Rubisco catalytic activity wherein the K_m for O_2 is significantly higher than a protein encoded by a parental polynucleotide encoding a naturally-occurring Rubisco enzyme or subunit.

18. A polynucleotide of claim 15, wherein the polynucleotide encodes an enhanced Rubisco protein having Rubisco catalytic activity wherein: (1) the K_m for CO_2 is significantly lower than a protein encoded by a parental polynucleotide encoding a naturally-occurring Rubisco enzyme, (2) the K_m for O_2 is significantly higher than a protein encoded by a parental polynucleotide encoding a naturally-occurring Rubisco enzyme, and/or (3) the ratio of the K_m for CO_2 to the K_m for O_2 is significantly lower than a protein encoded by a parental polynucleotide encoding a naturally-occurring Rubisco enzyme.

19. A method of producing a recombinant cell having an elevated carbon fixation activity, the method comprising:

(A) recombining one or more first Calvin or Krebs cycle enzyme coding nucleic acid, or a homologue thereof, with one or more first homologous nucleic acid to produce a library of recombinant first enzyme nucleic acid homologues;

(B) optionally repeating step (A) one or more times using one or more members of the library of recombinant first enzyme nucleic acid homologues as the one or more first enzyme coding nucleic acid which is active in the Calvin cycle, or the homologue thereof, or as the one or more first homologous nucleic acid, thereby producing a diversified library of recombinant first enzyme nucleic acid homologues;

(C) selecting the library of recombinant first enzyme nucleic acid homologues or the diversified library of recombinant first enzyme nucleic acid homologues for one or more of: an increased catalytic rate, an altered substrate specificity, and an increased ability of a cell expressing one or more members of the library to fix CO₂ when the one or more library members is expressed in the cell, thereby producing a selected library of recombinant first enzyme nucleic acid homologues; and,

(D) recursively repeating steps A-C one or more times, wherein the selected library of recombinant first enzyme nucleic acid homologues provides one or more of: the one or more first Calvin or Krebs cycle enzyme coding nucleic acid, the homologue thereof, or the one or more first homologous nucleic acid of step (A), wherein steps A-C are repeated until one or more members of the selected library produces an elevated carbon fixation level in a target recombinant cell when the one or more selected library member is expressed in the target cell, as compared to a carbon fixation activity of the target cell when the one or more selected library member is not expressed in the target cell.

20. The method of claim 1, wherein the one or more first Calvin or Krebs cycle enzyme, or the homologue thereof, or the one or more homologous first nucleic acid encodes a Rubisco enzyme, a Calvin cycle operon, or a homologue thereof.

21. The method of claim 19, wherein the recombining step is performed in vitro, in silico or in vivo, or a combination thereof.

22. The selected library of claim 19.

23. The one or more selected library member of claim 19.

24. The diversified library of claim 19.

25. The target recombinant cell of claim 19.

26. A plant comprising the target recombinant cell of claim 25.

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